(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 June 2005 (23.06.2005)

PCT

(10) International Publication Number
WO 2005/056534 A1

(51) International Patent Classification[7]: C07D 239/70

(21) International Application Number:
PCT/US2004/040329

(22) International Filing Date: 2 December 2004 (02.12.2004)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/526,449 2 December 2003 (02.12.2003) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2005/056534 A1

(54) Title: REFERENCE STANDARD FOR CHARACTERIZATION OF ROSUVASTATIN

(57) Abstract: Provided are rosuvastatin degradation products and their use as a reference standard (including reference marker) for analysis of rosuvastatin.

REFERENCE STANDARD FOR CHARACTERIZATION OF ROSUVASTATIN

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application No. 60/526,449 filed on December 2, 2003, the disclosure of which is incorporated by reference in its entirety herein

FIELD OF THE INVENTION

The present invention relates to rosuvastatin degradation products and their use as a reference standard for analysis of rosuvastatin.

BACKGROUND OF THE INVENTION

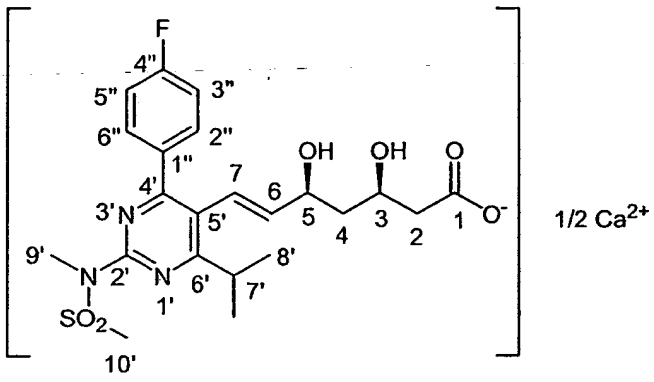
Statins are currently the most therapeutically effective drugs available for reducing low-density lipoprotein (LDL) particle concentration in the blood stream of patients at risk for cardiovascular disease. Thus, statins are used in the treatment of hypercholesterolemia, hyperlipoproteinemia, and atherosclerosis. A high level of LDL in the bloodstream has been linked to the formation of coronary lesions that obstruct the flow of blood and can rupture and promote thrombosis. Goodman and Gilman, The Pharmacological Basis of Therapeutics, page 879 (9th Ed. 1996).

Statins inhibit cholesterol biosynthesis in humans by competitively inhibiting the 3-hydroxy-3-methyl-glutaryl-coenzyme A ("HMG-CoA") reductase enzyme. HMG-CoA reductase catalyzes the conversion of HMG to mevalonate, which is the rate-determining step in the biosynthesis of cholesterol. Decreased production of cholesterol causes an increase in the number of LDL receptors and corresponding reduction in the concentration of LDL particles in the bloodstream. Reduction in the LDL level in the bloodstream reduces the risk of coronary artery disease. J.A.M.A. 1984, 251, 351-74.

Currently available statins include lovastatin, simvastatin, pravastatin, fluvastatin, cerivastatin and atorvastatin. Lovastatin (disclosed in U.S. Pat. No. 4,231,938) and simvastatin (disclosed in U.S. Pat. No. 4,444,784) are administered in the lactone form. After absorption, the lactone ring is opened in the liver by chemical or enzymatic hydrolysis, and the active hydroxy acid is generated.

Pravastatin (disclosed in U.S. Pat. No. 4,346,227) is administered as the sodium salt. Fluvastatin (disclosed in U.S. Pat. No. 4,739,073) and cerivastatin (disclosed in U.S. Pat. Nos. 5,006,530 and 5,177,080), also administered as the sodium salt, are entirely synthetic compounds that are in part structurally distinct from the fungal derivatives of this class that contain a hexahydronaphthalene ring. Atorvastatin and two new "superstatins," rosuvastatin and pitavastatin, are administered as calcium salts.

Rosuvastatin calcium (monocalcium bis (+) 7-[4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylaminopyrimidin)-5-yl]-(3R,5S)-dihydroxy-(E)-6-heptenoate) is an HMG-CoA reductase inhibitor, developed by Shionogi for the once daily oral treatment of hyperlipidaemia (Ann Rep, Shionogi, 1996; Direct communications, Shionogi, 8 Feb 1999 & 25 Feb 2000). Rosuvastatin calcium is a superstatin, which can lower LDL-cholesterol and triglycerides more effectively than first generation drugs. Rosuvastatin calcium has the following chemical formula:



Rosuvastatin calcium is marketed under the name CRESTOR for treatment of a mammal such as a human. According to the maker of CRESTOR, it is administered in a daily dose of from about 5mg to about 40 mg. For patients requiring less aggressive LDL-C reductions or who have pre-disposing factors for myopathy, the 5mg dose is recommended, while 10 mg dose is recommended for the average patient, 20 mg dose for patients with marked hyper-cholesterolemia and aggressive lipid targets (>190 mg/dL), and the 40 mg dose for patients who have not been responsive to lower doses.

U.S. Pat. No. 5,260,440 discloses and claims rosuvastatin, its calcium salt (2:1), and its lactone form. The process of the '440 patent prepares rosuvastatin by reacting 4-(4-fluorophenyl)-6-isopropyl-2-(N-methyl-N-methylsulfonylamino)-5-

pyrimidinecarbaldehyde with methyl (3R)-3-(tert-butyldimethylsilyloxy)-5-oxo-6-triphenylphosphoranylidene hexanate in acetonitrile under reflux. The silyl group is then cleaved with hydrogen fluoride, followed by reduction with $NaBH_4$ and diethylmethoxyborane in THF to obtain a methyl ester of rosuvastatin.

The ester is then hydrolyzed with sodium hydroxide in ethanol at room temperature, followed by removal of ethanol and addition of ether, to obtain the sodium salt of rosuvastatin. The sodium salt is then converted to the calcium salt. The sodium salt is dissolved in water and maintained under a nitrogen atmosphere. Calcium chloride is then added to the solution, resulting in precipitation of rosuvastatin calcium (2:1). The process for preparation of the intermediates disclosed in the '440 patent is incorporated herein by reference.

U.S. Pat. No. 6,316,460 discloses a pharmaceutical composition of rosuvastatin. The pharmaceutical compositions contain rosuvastatin or its salt and a multivalent tribasic phosphate salt.

The product mixture of a reaction rarely is a single compound pure enough to comply with pharmaceutical standards. Side products and byproducts of the reaction and adjunct reagents used in the reaction will, in most cases, be present. At certain stages during processing of the rosuvastatin contained in the product mixture into an active pharmaceutical ingredient ("API"), the rosuvastatin must be analyzed for purity, typically by HPLC or GC analysis, to determine if it is suitable for continued processing or ultimately for use in a pharmaceutical product. The rosuvastatin does not need to be absolutely pure. Absolute purity is a theoretical ideal that is unattainable. Rather, there are purity standards intended to ensure that an API is not made less safe for clinical use because of the presence of impurities. In the United States, the Food and Drug Administration guidelines recommend that applicants limit some impurities to below 0.1%.

Generally, side products, byproducts and adjunct reagents (collectively "impurities") are identified spectroscopically and by other physical methods and then the impurities are associated with a peak position in a chromatogram (or a spot on a TLC plate). (Strobel p. 953) (Strobel, H.A.; Heineman, W.R., *Chemical Instrumentation: A Systematic Approach, 3rd dd.* (Wiley & Sons: New York 1989)). Thereafter, the impurity can be identified by its position in the chromatogram, which is conventionally measured in minutes between injection of the sample on the column and elution of the particular component through the detector, known as the "retention

time." This time period varies daily based upon the condition of the instrumentation and many other factors. To mitigate the effect that such variations have upon accurate identification of an impurity, practitioners use "relative retention time" ("RRT") to identify impurities. (Strobel p. 922). The RRT of an impurity is its retention time divided by the retention time of some reference marker. In theory, rosuvastatin itself could be used as the reference marker, but as a practical matter it is present in such overwhelming proportion in the mixture that it tends to saturate the column, leading to irreproducible retention times, *i.e.*, the maximum of the peak corresponding to rosuvastatin tends to wander (Strobel Fig. 24.8(b) p. 879, contains an illustration of the sort of asymmetric peak that is observed when a column is overloaded). Thus, it is sometimes desirable to select an alternative compound that is added to, or is present in, the mixture in an amount significant enough to be detectable and sufficiently low as not to saturate the column and to use that compound as the reference marker.

Researchers and developers in drug manufacturing understand that a compound in a relatively pure state can be used as a "reference standard" (a "reference marker" is similar to a reference standard but it is used for qualitative analysis) to quantify the amount of the compound in an unknown mixture. When the compound is used as an "external standard," a solution of a known concentration of the compound is analyzed by the same technique as the unknown mixture. (Strobel p. 924, Snyder p. 549) (Snyder, L.R.; Kirkland, J.J. *Introduction to Modern Liquid Chromatography, 2nd ed.* (John Wiley & Sons: New York 1979)). The amount of the compound in the mixture can be determined by comparing the magnitude of the detector response. *See also* USP 6,333,198, incorporated herein by reference.

The reference standard compound also can be used to quantify the amount of another compound in the mixture if the "response factor," which compensates for differences in the sensitivity of the detector to the two compounds, has been predetermined. (Strobel p. 894). For this purpose, the reference standard compound may be added directly to the mixture, in which case it is called an "internal standard." (Strobel p. 925, Snyder p. 552).

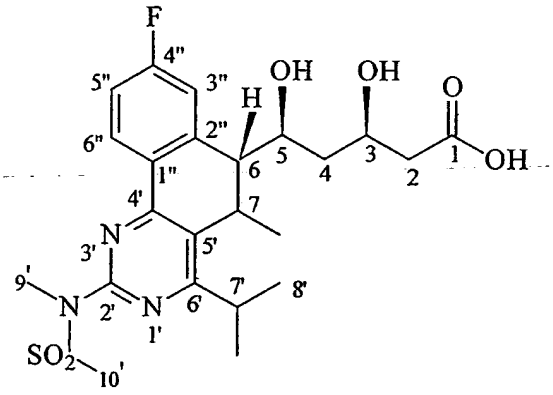
The reference standard compound can even be used as an internal standard when the unknown mixture contains some of the reference standard compound by using a technique called "standard addition," wherein at least two samples are prepared by adding known and differing amounts of the internal standard. (Strobel

pp. 391-393, Snyder pp. 571, 572). The proportion of detector response due to the reference standard compound that is originally in the mixture can be determined by extrapolation of a plot of detector response versus the amount of the reference standard compound that was added to each of the samples to zero. (e.g. Strobel, Fig. 11.4 p. 392).

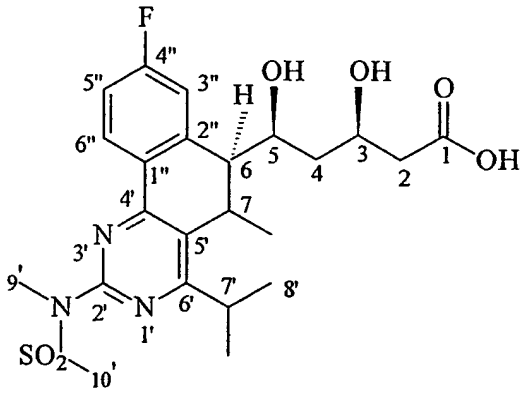
The present invention provides a rosuvastatin degradation product that can be used as a reference standard for analysis of rosuvastatin.

SUMMARY OF THE INVENTION

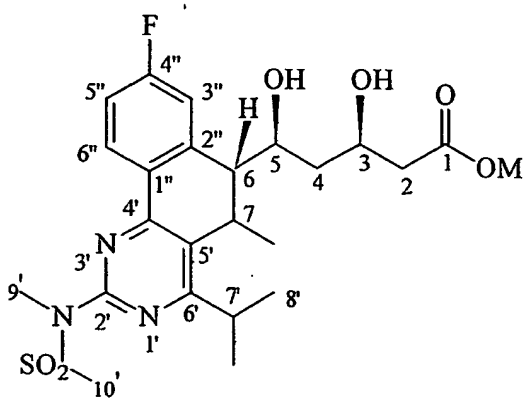
In one aspect, the present invention provides a rosuvastatin degradation product having the following structure:



In another aspect, the present invention provides a rosuvastatin degradation product having the following structure:

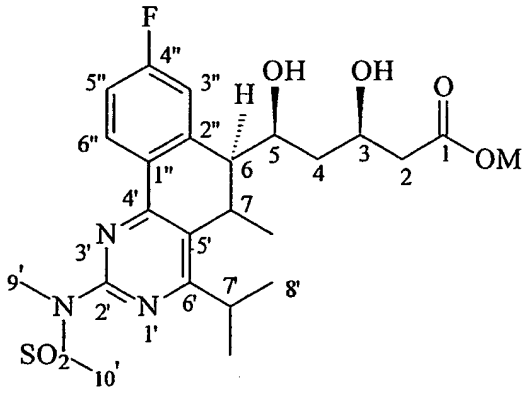


In another aspect, the present invention provides a rosuvastatin degradation product having the following structure:



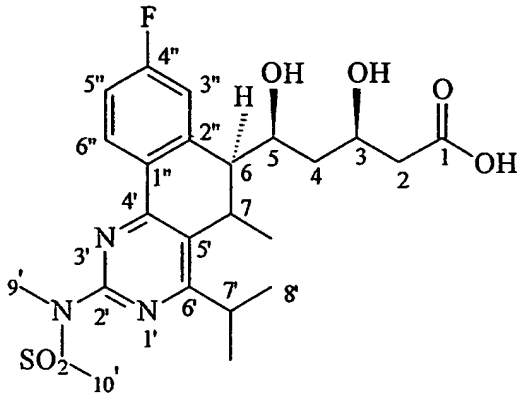
wherein M is an alkali or alkaline earth metal.

In another aspect, the present invention provides a rosuvastatin degradation product having the following structure:

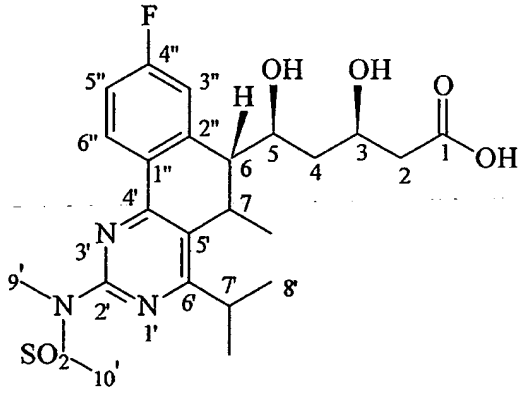


wherein M is an alkali or alkaline earth metal. Preferably M is calcium. The calcium salt may be converted to lactone form by combining acetonitrile, hydrochloric acid and the calcium salt to obtain the lactone; or to free acid comprising dissolving the calcium salt in a mixture of acetonitrile and water, and contacting the calcium salt with a silica column.

In another aspect the present invention provides a lactone form of a rosuvastatin degradation product having the following structure:



In another aspect, the present invention provides a lactone form of a rosuvastatin degradation product having the following structure:



In another aspect, the present invention provides a process for converting the lactone to a calcium salt comprising hydrolyzing the lactone under aqueous basic conditions, and reacting the hydrolyzed lactone with a source of calcium.

In another aspect, the present invention provides a process for converting the lactone to free acid form comprising hydrolyzing the lactone under aqueous basic conditions to obtain a metal salt and contacting the metal salt with a silica column.

Preferably the degradation product is about 95% free by weight of its corresponding stereoisomer at position 6. The rosuvastatin degradation product may be isolated or purified.

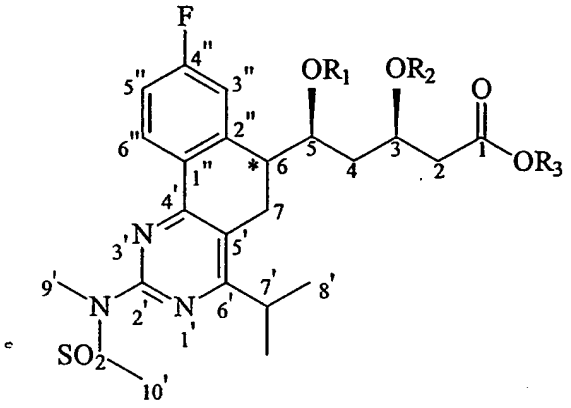
In another aspect, the present invention provides a method for analyzing a sample of rosuvastatin comprising the steps of:

a) performing chromatography on the sample to obtain data; and

b) comparing the data with the chromatography data of the degradation product.

In another aspect the present invention provides a process for preparing the degradation product comprising the step of irradiating with visible light rosuvastatin acid, rosuvastatin alkali or alkaline earth metal salt or rosuvastatin lactone.

In another aspect, the present invention provides a method for determining the retention time of a chromatography column for rosuvastatin, comprising the steps of carrying out chromatography with the following compound as a standard,



wherein R_1 and R_2 are independently hydrogen or a hydrolyzable protecting group; R_3 is hydrogen, a C_1 to C_4 alkyl group, or an alkali or alkaline earth metal; or wherein C^1 and C^5 form a lactone.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is an HPLC chromatogram of Compound VI.

Figure 2 is an HPLC chromatogram of Compound VII.

Figure 3 is an HPLC chromatogram of a mixture of rosuvastatin, Compound VI, and Compound VII.

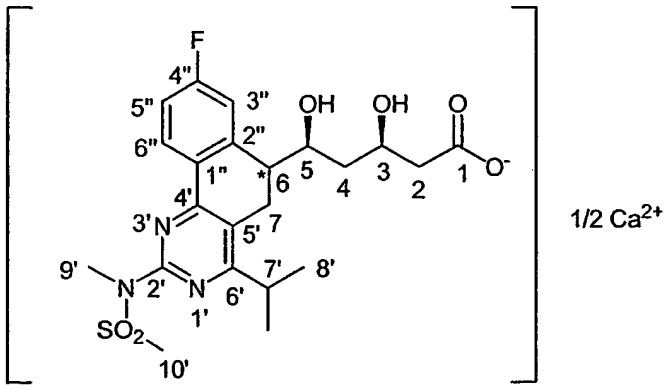
DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "reference standard" refers to a compound that may be used both for quantitative and qualitative analysis of an active pharmaceutical ingredient. For example, the retention time of the compound in HPLC allows for setting a relative retention time, thus making qualitative analysis possible. The concentration of the compound in solution before injection into an HPLC column allows for comparison of the areas under the peaks in an HPLC chromatogram, thus making quantitative analysis possible.

A "reference marker" is used in qualitative analysis to identify components of a mixture based upon their position, e.g. in a chromatogram or on a Thin Layer Chromatography (TLC) plate (Strobel pp. 921, 922, 953). For this purpose, the compound does not necessarily have to be added to the mixture if it is present in the mixture. A "reference marker" is used only for qualitative analysis, while a reference standard may be used for quantitative or qualitative analysis, or both. Hence, a reference marker is a subset of a reference standard, and is included within the definition of a reference standard.

Although some of the knowledge of those in the art regarding reference standards has been described in general terms up to this point, those skilled in the art also understand that the detector response can be, for example, the peak heights or integrated peak areas of a chromatogram obtained, e.g. by UV or refractive index detection, from the eluent of an HPLC system or, e.g. flame ionization detection or thermal conductivity detection, from the eluent of a gas chromatograph, or other detector response, e.g. the UV absorbence, of spots on a fluorescent TLC plate. The position of the reference standard may be used to calculate the relative retention time for rosuvastatin and other impurities.

When rosuvastatin calcium is exposed to visible light irradiation, degradation products of rosuvastatin are obtained, which can be used as a reference standard. The two degradation products are diastereomeric cyclic products (II) and (III) with the creation of an additional asymmetric center in position 6 as follows:



In addition to rosuvastatin calcium, other forms of rosuvastatin may be irradiated, including the lactone, free acid and salts such as sodium salt.

The irradiation may be performed in solution or in solid state. When irradiating a solution, the irradiation may be performed at preferably from about room

temperature up to about reflux temperature. The organic solvent used for dissolution may be either polar protonic (C_1 to C_4 alcohol such as methanol or ethanol) or aprotonic (acetonitrile, tetrahydrofuran) in a mixture with water. Visible light irradiation of about 750w at about 35°C of aqueous acetonitrile solution of rosuvastatin calcium for about 7 hours gives a mixture of compounds (II and III) in the ratio 1:1. When irradiating in the solid state, the temperature is preferably from about 20EC to about 100EC. One of skill in the art may yet choose a narrow spectrum within these spectrums or a mixture of various spectrums. Based on the structural guidance provided herein of the various degradation products, one of skill in the art may prepare a synthetic route to obtain the degradation products.

In another embodiment, the corresponding lactone is obtained, either by irradiating the lactone form of rosuvastatin or by preparing the lactone from Compounds II and III to obtain the corresponding Compounds IV and V which can be used as a reference standard. Compound IV has the following ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 1.24, 1.34, 1.68, 2.47, 2.53, 2.64, 3.15, 3.35, 3.46, 3.56, 3.60, 4.28, 4.45, 6.99, 7.14, 8.31; ^{13}C NMR (75MHz, $CDCl_3$) δ (ppm): 20.87, 21.26, 23.29, 31.41, 33.26, 34.03, 38.23, 42.07, 43.00, 62.23, 74.53, 115.61, 116.08 (J=26Hz), 116.25 (J=22Hz), 129.08, 128.91 (J=9Hz), 139.28 (J=8Hz), 157.64, 157.86, 163.96 (J=253Hz), 169.47, 174.48; FAB+m/z (MH^+): 464. Compound V has the following ^{1}H NMR (300MHz, $CDCl_3$) δ (ppm): 1.24, 1.29, 1.52, 1.70, 2.58, 3.02, 3.21, 3.27, 3.41, 3.55, 3.60, 4.26, 4.78, 7.05, 7.12, 8.34; FAB+m/z (MH^+): 464.

The preparation of the corresponding lactone compounds IV and V from compound II and III includes dissolving compound II and III in a suitable solvent and forming a lactone ring for example with aqueous hydrochloric acid. Other acids may be used to form a lactone. A suitable solvent for preparation of the lactone from Compound II or III is dichloromethane, chloroform, acetonitrile or tetrahydrofuran. Preferably, the suitable solvent is acetonitrile. To recover the product, the solvent may be removed by any conventional process, such as evaporation. Obtained compounds IV and V can be separated by methods such as column chromatography and crystallization.

The preparation of the corresponding lactone compounds IV and V from rosuvastatin lactone may also be performed by visible light irradiation in the same solvents. The irradiation characteristics are as described above. The lactone

compounds IV and V may be hydrolyzed with an equivalent amount of an aqueous base such as sodium or calcium hydroxide to obtain the corresponding salts in the presence of a solvent. Preferably, the solvent is acetonitrile. In one embodiment, the lactones are hydrolyzed with an aqueous solution of sodium hydroxide in acetonitrile, followed by removal of acetonitrile and addition of a source of calcium such as calcium chloride.

The lactone forms IV and V can be hydrolyzed with a base to obtain salts and thereafter converted to acid forms VI and VII, respectively, which can be used as a reference standard. The conversion of the lactones to salts may be carried out by using a basic aqueous solution. In one embodiment, the lactone is dissolved in a mixture of acetonitrile and an aqueous NaOH solution. The acetonitrile is then removed and a source of calcium such as calcium chloride is added to precipitate the calcium salt.

The acid forms are obtained after purifying the salt products by column chromatography on silica gel (as described in example 1). It is believed that the acidity of the silica column is responsible for the conversion. Compound VI has the following ^{1}H NMR (600MHz, $CDCl_3$+CD_3OD 5:1) δ (ppm): 1.25, 1.33, 1.39, 1.58, 2.22, 2.80, 2.89, 3.36, 3.52, 3.58, 3.61, 3.95, 7.01, 7.09, 8.26; ^{13}C NMR (150MHz, $CDCl_3$+CD_3OD 5:1) δ (ppm): 21.14, 21.31, 23.32, 31.56, 33.47, 40.15, 42.16, 42.97, 44.62, 68.99, 71.45, 115.17 (J=21Hz), 116.32 (J=22Hz), 117.77, 128.87 (J=9Hz), 129.20, 142.34 (J=8Hz), 157.71, 158.57, 164.26 (J=251Hz), 174.30; Cl+m/z (MH^+): 482. Compound VII has the following ^{1}H NMR (600MHz, $CDCl_3$+CD_3OD 5:1) δ (ppm): 1.24, 1.31, 1.43, 2.25, 2.95, 3.05, 3.19, 3.30, 3.56, 3.60, 3.85, 4.03, 7.02, 7.08, 7.31; ^{13}C NMR (150MHz, $CDCl_3$+CD_3OD 5:1) δ (ppm): 21.15, 21.23, 22.98, 31.22, 33.36, 38.83, 42.08, 43.45, 68.82, 73.86, 114.95 (J=21Hz), 116.31 (J=21Hz), 117.41, 128.56 (J=8Hz), 128.91, 142.02 (J=8Hz), 157.58, 158.45, 164.28 (J=252Hz), 173.19, 178; Cl+m/z(MH^+): 482.

The various forms of the degradation product may be purified so that only one stereoisomer is present. The R stereoisomer at position 6 is preferably at least about 95% free of the S stereoisomer by weight. Conversely, the S stereoisomer at position 6 is preferably at least about 95% free of the R stereoisomer by weight. Purification may be performed by column chromatography, TLC, HPLC, or other known purification methods.

Instruments

For chromatography, aluminum oxide or, preferably, silica gel may be used for packing. As for the eluent, different organic solvents or mixtures thereof may be used. Ethyl acetate is preferred.

Compounds II and III, isolated as corresponding acids (VI and VII), lactones (IV and V) can be investigated with ^{1}H NMR, ^{13}C NMR, COSY NMR and mass spectroscopic analyses to determine their structures.

EXAMPLES

Example 1. Preparation of rosuvastatin degradation products by irradiation of rosuvastatin (Ca salt).

1. Rosuvastatin (Ca-salt) (4.0g) was dissolved in a mixture acetonitrile-water (380ml-140ml) and irradiated with visible light (750w, 35°C) for 7 hours. Acetonitrile and water were evaporated under vacuum.

2. The obtained solid was dissolved in 40ml of acetonitrile and 40ml of 1N hydrochloric acid was added. The mixture was stirred at room temperature overnight. After evaporation of acetonitrile and water, and drying under vacuum, the obtained products were separated by column chromatography on silica gel (eluent hexane-ethyl acetate 1:2), giving lactone IV (0.8g) and lactone V (0.6g). TLC on silica gel, eluent hexane-ethyl acetate (1:2) R_f=0.30 for Compound IV, R_f=0.25 for Compound V.

Compound IV

Number atom	^{1}H NMR ($CDCl_3$)	^{13}C NMR ($CDCl_3$)	
	Δ	Δ	*J*(Hz)
1		169.47	
2	2.53, 2.64	38.23	
3	4.28	62.23	
4	1.68	34.03	
5	4.45	74.53	
6	3.15	43.00	
7	2.47, 3.46	23.29	
2'		157.64	
4'		157.86	
5'		115.61	
6'		174.48	
7'	3.35	31.41	
8'	1.24, 1.34	21.36, 20.87	
9'	3.60	33.26	
10'	3.56	42.07	
1"		129.08	
2"		139.28	8
3"	6.99	116.25	22
4"		163.96	253
5"	7.14	116.08	26
6"	8.31	128.91	9

Compound V

Number atom	^{1}H NMR ($CDCl_3$)
	Δ
2	2.58
3	4.26
4	1.52, 1.70
5	4.78
6	3.41
7	3.02, 3.21
7'	3.27
8'	1.24, 1.29
9'	3.60
10'	3.55
3"	7.05
4"	
5"	7.12
6"	8.34

3. Lactone IV (0.8g) was dissolved in acetonitrile, and 1N aqueous sodium hydroxide (4ml) was added. The mixture was stirred at room temperature overnight. After evaporation of acetonitrile and water, and drying under vacuum, the obtained product

was purified by column chromatography on silica gel (eluent dichloromethane-methanol 65ml:10ml), giving pure Compound VI (0.4g).

Compound VI (Corresponding acid of Compound IV)

Number atom	^{1}H NMR ($CDCl_3$+CD_3OD 5:1)	^{13}C NMR ($CDCl_3$+CD_3OD 5:1)	
	Δ	Δ	*J* (Hz)
1			
2	2.22	42.97	
3	3.95	68.99	
4	1.39/1.58	40.15	
5	3.58	71.45	
6	2.89	44.62	
7	2.80/3.52	23.32	
2'		157.71	
4'		158.57	
5'		117.77	
6'		174.30	
7'	3.36	31.56	
8'	1.25/1.33	21.14/21.31	
9'	3.61	33.47	
10'	3.58	42.16	
1"		129.20	
2"		142.34	8
3"	7.01	116.32	22
4"		164.26	251
5"	7.09	115.17	21
6"	8.26	128.87	9

4. Analogously, Compound VII (0.3g) was obtained from lactone V.

Compound VII (Corresponding acid of Compound V)

Number atom	^{1}H NMR ($CDCl_3$+CD_3OD 5:1)	^{13}C NMR ($CDCl_3$+CD_3OD 5:1)	
	Δ	Δ	*J*(Hz)
1		178	
2	2.25	42.08	
3	4.03	68.82	
4	1.43	38.83	
5	3.85	73.86	
6	3.05	43.45	
7	2.95/3.19	22.98	
2'		157.58	
4'		158.45	
5'		117.41	
6'		173.19	
7'	3.30	31.22	
8'	1.24/1.31	21.15/21.23	
9'	3.60	33.36	
10'	3.56	42.08	
1"		128.91	
2"		142.02	8
3"	7.02	116.31	21
4"		164.28	252
5"	7.08	114.95	21
6"	8.31	128.56	8

Example 2. Preparation of rosuvastatin degradation products by irradiation of rosuvastatin lactone

1. Rosuvastatin lactone (2.0g) was dissolved in 200ml of acetonitrile and irradiated with visible light (750w, 35°C) for 7 hours. After evaporation of the acetonitrile, and drying under vacuum, the obtained products were separated by column chromatography on silica gel (eluent hexane-ethyl acetate 1:2), giving lactone IV (1.1g) and lactone V (0.6g).

2. Lactone IV (1.0g) was dissolved in 5ml of acetonitrile and 2ml of 1N aqueous NaOH was added. The mixture was stirred at room temperature for 4 hours. After evaporation of acetonitrile, 1ml of 2N aqueous $CaCl_2$ was added, and the mixture was stirred for 1 hour at room temperature. The precipitate was filtered and dried under vacuum giving Compound II.

3. Analogously, Compound III was obtained from lactone V.

Example 3. HPLC impurity profile determination of rosuvastatin calcium

The purity of Compounds IV, V, VI and VII is determined by HPLC analysis.

HPLC

Column: C18

Eluent: Gradient of Formate buffer and Acetonitrile

Flow: 1ml/min

Detector: 245nm

Sample volume: 10ul

Diluent: Acetonitrile: Water = 50:50

Mobile phase composition and flow rate may be varied in order to achieve the required system suitability.

Sample Preparation

About 10mg of rosuvastatin calcium sample is weighed in a 20ml amber volumetric flask. The sample is dissolved with 10ml acetonitrile and diluted to volume with water.

Standard Preparation

About 10mg of each Compounds IV, V, VI and VII are weighed in a 20ml amber volumetric flask, dissolved with 10ml acetonitrile and diluted to volume with water. 1ml of prepared solution is diluted to 100ml with diluent.

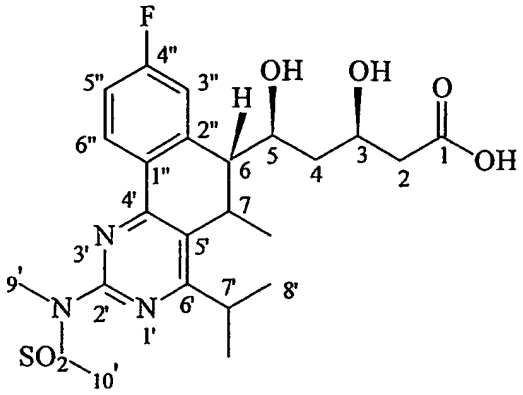
Method

The freshly prepared sample solutions are injected into the chromatograph, and the chromatogram of the sample is continued up to the end of the gradient. The areas for each peak in each solution is determined using a suitable integrator.

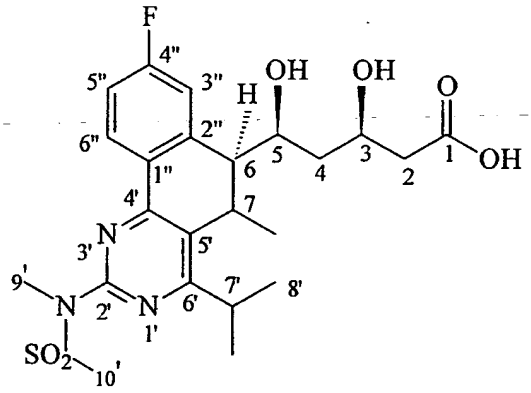
Having thus described the invention with reference to particular preferred embodiments and illustrated it with Examples, those in the art can appreciate modifications to the invention as described and illustrated that do not depart from the spirit and scope of the invention as disclosed in the specification. The Examples are set forth to aid in understanding the invention but are not intended to, and should not be construed to, limit its scope in any way. The examples do not include detailed descriptions of conventional methods. All references mentioned herein are incorporated in their entirety.

WHAT IS CLAIMED IS:

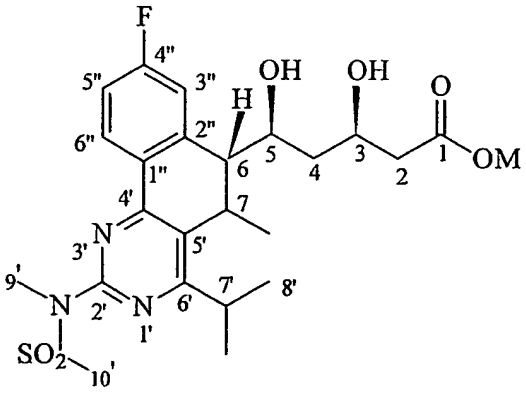
1. A rosuvastatin degradation product having the following structure:



2. A rosuvastatin degradation product having the following structure:

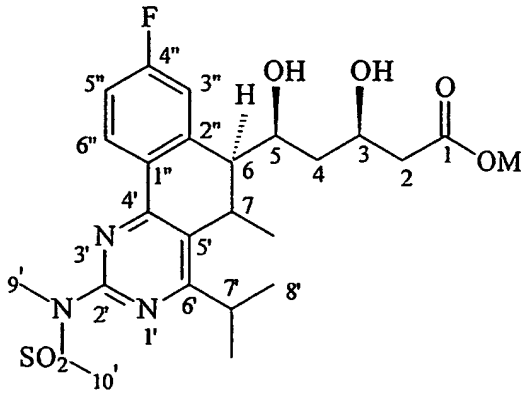


3. A rosuvastatin degradation product having the following structure:



wherein M is an alkali or alkaline earth metal.

4. A rosuvastatin degradation product having the following structure:



wherein M is an alkali or alkaline earth metal.

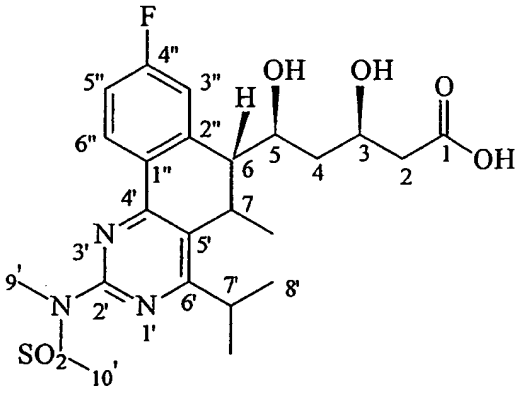
5. The rosuvastatin degradation product of claim 3 or 4, wherein M is calcium.

6. A process for converting the calcium salt of claim 5 to lactone form comprising combining acetonitrile, hydrochloric acid and the calcium salt to obtain the lactone.

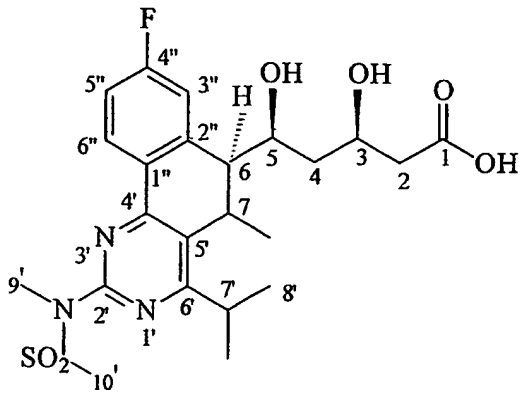
7. A process for converting the calcium salt of claim 5 to a free acid comprising dissolving the calcium salt in a mixture of acetonitrile and water to obtain a solution, and contacting the solution with a silica column.

8. The process of claim 7, wherein the process comprises evaporating the solution to obtain a residue, dissolving the residue in a mixture of water and acetonitrile to obtain a second solution, injecting the second solution into a silica column and eluting the column with a mixture of dichloromethane and methanol.

9. A lactone form of a rosuvastatin degradation product having the following structure:



10. A lactone form of a rosuvastatin degradation product having the following structure:



11. A process for converting the lactone of claim **9** or **10** to a calcium salt comprising hydrolyzing the lactone under aqueous basic conditions, and reacting the hydrolyzed lactone with a source of calcium.

12. The process of claim **11**, wherein a basic aqueous solution is combined with a solution of the lactone in acetonitrile, followed by removal of acetonitrile and addition of a source of calcium, and recovery of the calcium salt.

13. The process of claim **12**, wherein the process comprises dissolving the lactone in a mixture of acetonitrile and aqueous sodium hydroxide, evaporating the acetonitrile, adding calcium chloride to the remaining water to precipitate the calcium salt.

14. A process for converting the lactone of claim **9** or **10** to free acid form comprising hydrolyzing the lactone under aqueous basic conditions to obtain a salt and contacting the salt with a silica column to obtain the free acid.

15. The rosuvastatin degradation product of Claim **1, 2, 3, 4, 9** or **10**, wherein the degradation product is about 95% free by weight of its corresponding stereoisomer at position 6.

16. The rosuvastatin degradation product of claim **1, 2, 3, 4, 9** or **10**, wherein the rosuvastatin degradation product is isolated or purified.

17. A method for analyzing a sample of rosuvastatin comprising the steps of:

a) performing chromatography on the sample to obtain data; and

b) comparing the data with the chromatography data of the degradation product of claim **1, 2, 3, 4, 9** or **10**.

18. The method of Claim **17**, wherein the method comprises the following steps:

(a) preparing a solution of rosuvastatin containing the degradation product;

(b) subjecting the solution to a high pressure liquid chromatography to obtain a chromatogram; and

(c) comparing a peak obtained in the chromatogram to a peak resulting from the degradation product.

19. The method of Claim **17**, wherein the method comprises the following steps:

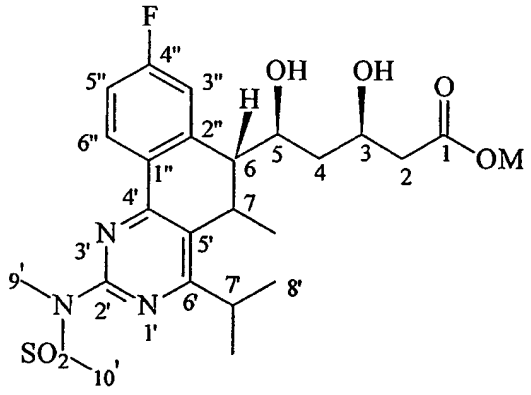
(a) preparing a solution of rosuvastatin containing the degradation product;

(b) subjecting the solution to thin layer chromatography to obtain a chromatogram; and

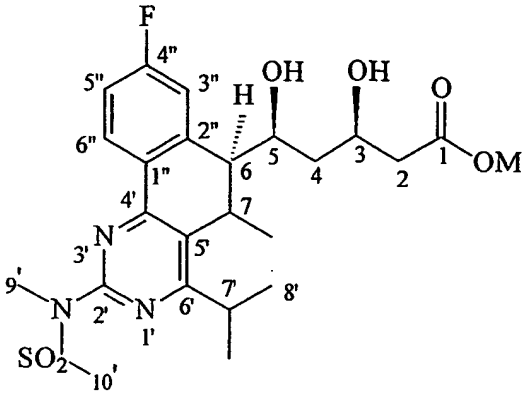
(c) comparing a band or spot obtained in the chromatogram to a peak or band resulting from the degradation product.

20. A process for preparing the degradation product of claim **1, 2, 3, 4, 9 or 10** comprising the step of irradiating with visible light rosuvastatin acid, rosuvastatin alkali or alkaline earth metal salt or rosuvastatin lactone.

21. A process of for preparing the compound:



or

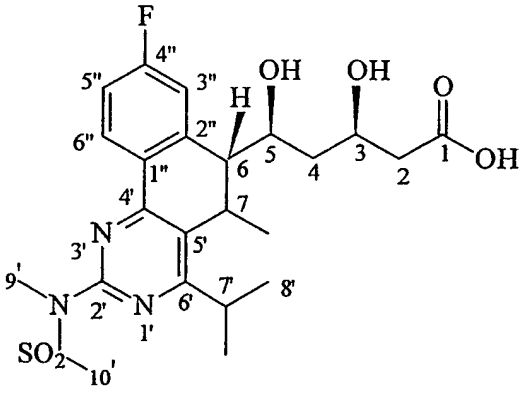


wherein M is calcium, comprising irradiating with visible light rosuvastatin calcium in solution in a mixture of an organic solvent and water.

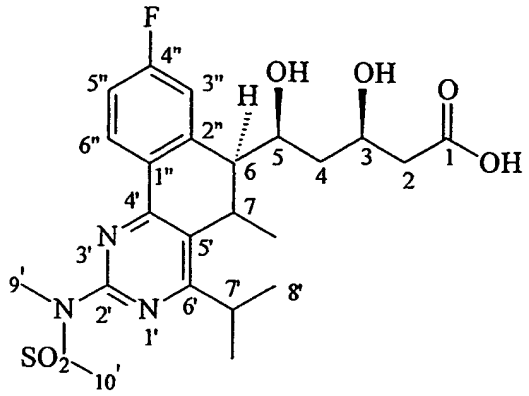
22. The process of claim **21**, wherein the organic solvent is acetonitrile.

23. The process of claim **21**, wherein the visible light radiation is of about 750w at about 35°C.

24. A process of for preparing lactone form of compound



or

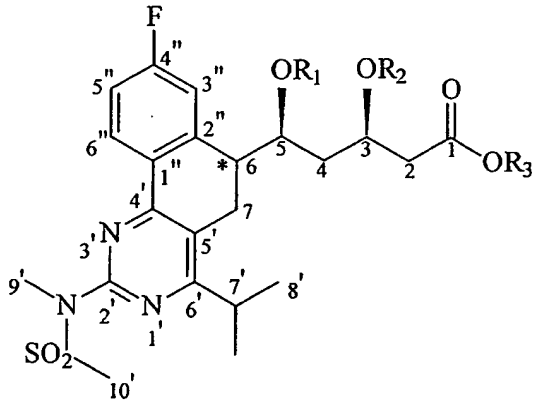


comprising irradiating with visible light rosuvastatin lactone in a solvent.

25. The process of claim **24**, wherein the visible light radiation is at about 20°C to about 100°C.

26. The process of claim **24**, wherein the solvent is acetonitrile.

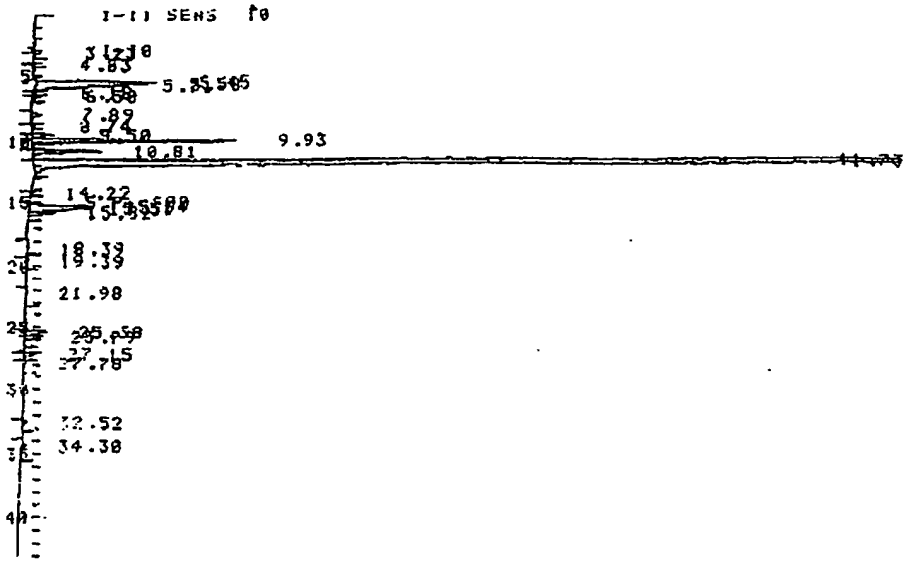
27. A method for determining the retention time of a chromatography column for rosuvastatin, comprising the steps of carrying out chromatography with the following compound as a standard,



wherein R_1 and R_2 are independently hydrogen or a hydrolyzable protecting group; R_3 is hydrogen, a C_1 to C_4 alkyl group, or an alkali or alkaline earth metal; or wherein C^1 and C^5 form a lactone.

28. The process of claim **27**, wherein the reference standard is a reference marker.

Figure 1. HPLC chromatogram of Compound VI



0-2500

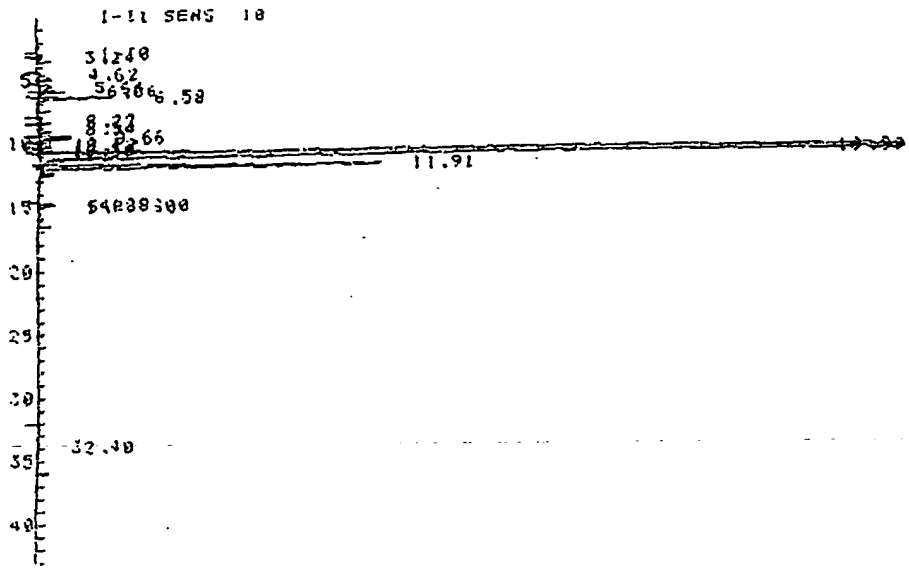
METHOD: ROSUVASTATIN TAB: 11 CH: 1

FILE: 0 CALC METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	HEIGHT	CONC
1	3.23	1420	289	0.042
2	4.03	881	112	0.026
3	5.45	36038	4611	1.060
4	5.59	34647	4268	1.019
5	5.71	36498	3199	1.074
6	6.18	711	90	0.021
7	6.58	3533	363	0.104
8	7.89	5746	271	0.169
9	8.74	4156	186	0.122
10	9.50	9231	918	0.272
11	9.93	81278	7883	2.391
12	10.81	30805	2695	0.906
13	11.72	3045450	292006	89.606
14	14.22	1604	169	0.047
15	15.34	30955	2471	0.911
16	15.51	20458	1902	0.602
17	15.82	12588	1044	0.370
18	18.39	2461	95	0.072
19	19.53	2994	124	0.088
20	21.98	1153	54	0.034
21	25.58	12415	889	0.365
22	25.79	10204	608	0.300
23	27.15	7179	489	0.211
24	27.79	2020	133	0.059
25	32.52	2259	200	0.066
26	34.30	2036	192	0.060
TOTAL		3398703	325245	100.000

PEAK REJ : 0

Figure 2. HPLC chromalogram of Compound VII



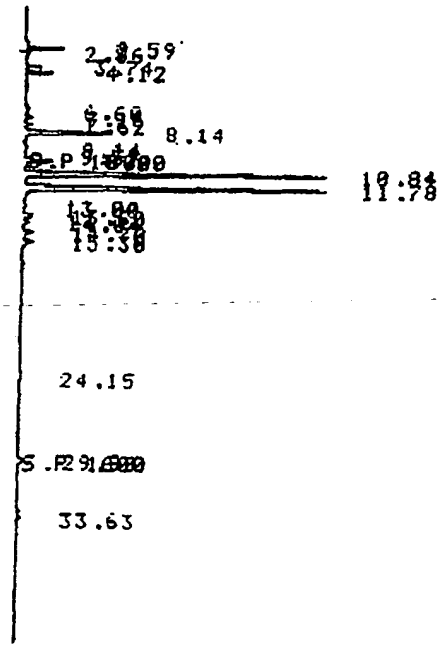
D-2500

METHOD: ROSUVASTATIN TAG: 12 CH: 1

FILE: 8 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

NO.	RT	AREA	HEIGHT	CONC
1	3.24	382	199	0.012
2	4.62	2378	382	0.077
3	5.64	10401	497	0.336
4	6.06	8915	1025	0.288
5	6.58	25006	1207	0.809
6	8.22	3226	130	0.104
7	8.94	2690	174	0.087
8	9.66	12439	1322	0.404
9	10.12	1914	196	0.062
10	10.58	1922	171	0.062
11	10.99	2871099	281993	92.854
12	11.91	137161	13416	4.436
13	14.88	9018	593	0.292
14	32.40	5465	74	0.177
TOTAL		3092949	303136	100.000
PEAK REJ :		0		

Rosuvastatin, Compound VI, Compound VII



0

): ROSUVASTATIN TAG: 12 CH: 1

0 CALC-METHOD: AREA% TABLE: 0 CONC: AREA

RT	AREA	CONC	BC
2.59	18433	3.611	BB
4.12	10807	2.117	BB
8.14	53524	10.485	VB
10.00	20230	3.963	BB
10.84	199132	39.008	BB
11.78	208366	40.817	BB
	510492	100.000	

:EJ # 10000

INTERNATIONAL SEARCH REPORT

Intern. Application No
PCT/US2004/040329

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07D239/70

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HULL C K ET AL: "Quantification of rosuvastatin in human plasma by automated solid-phase extraction using tandem mass spectrometric detection" JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL SCIENCES & APPLICATIONS, ELSEVIER SCIENCE PUBLISHERS, NL, vol. 772, no. 2, 5 June 2002 (2002-06-05), pages 219-228, XP004352369 ISSN: 1570-0232 the whole document	28

☐ Further documents are listed in the continuation of box C.

☐ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
30 March 2005	06/04/2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Usuelli, A